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Sensor system for *in-situ* and real-time monitoring of polymer (bio)degradation

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Abstract

A sensor system for investigating (bio)degradation processes of polymers is presented. The system utilizes semiconductor field-effect sensors and is capable of monitoring the degradation process *in-situ* and in real-time. The degradation of the polymer poly(D,L-lactic acid) is exemplarily monitored in solutions with different pH value, pH-buffer solution containing the model enzyme lipase from *Rhizomucor miehei* and cell-culture medium containing supernatants from stimulated and non-stimulated THP-1-derived macrophages mimicking activation of the immune system.

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Keywords: Field-effect sensor; enzymatic (bio)degradation; cell activity; poly(D,L-lactic acid); *in-situ* monitoring; impedance spectrscopy

1. Introduction

(Bio)degradable polymers have become very important for the field of medical applications. Their ability to disappear after a certain period of time or under specific conditions has stimulated the creation of a large number of biomedical devices such as temporary implants [1–4], scaffolds for tissue engineering [1,5,6], or drug-release systems [7,8]. Nevertheless, with regard to precise prediction of the degradation process, a large number of parameters need to be studied concerning their impact on the degradation process. This is of particular relevance

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with regard to the composition of the biological environment, in which degradation takes place. Due to the interaction with enzymes and other substances secreted from cells, there is the risk of unintended acceleration of the degradation process.

Recently, a novel sensor system based on field-effect electrolyte-insulator-semiconductor devices has been presented that enables *in-situ* monitoring of polymer (bio)degradation [9,10]. The setup of this system is schematically depicted in Fig. 1. Due to its capability of real-time measurement and option to monitor multiple devices in parallel, the system provides distinct potential to gain higher throughput of samples in degradation studies. In this work, the applicability of the sensor system for monitoring of polymer (bio)degradation in different degradation media is demonstrated. A commercial biodegradable polymer of the type poly(D,L-lactic acid) (PDLLA) has been used as model system.

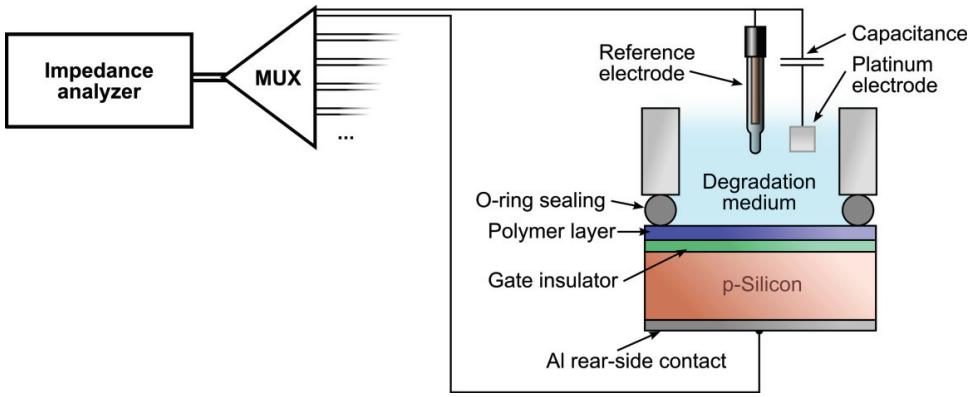

Fig. 1. Schematic of the sensor structure and measurement setup. The impedance analyser is connected via a multiplexer (MUX) to several polymer-modified electrolyte–insulator–semiconductor (PMEIS) sensors.

2. Materials and Methods

Poly(lactic acid) was selected as a model polymer because of its outstanding importance as (bio)degradable material for biomedical applications. The used poly(lactic acid)-type polymer (Resomer® R202H, Evonik Röhm GmbH, Germany) has an isotactic order of the two enantiomers L- and D-lactic acid and a molar mass in the range of M = 10,000 – 18,000 g/mol. The PDLLA was solved in methyl ethyl ketone and spin-coated on the surface of several sensors resulting in thin polymer layers of about 500 nm thickness. The thickness has been investigated by profilometry; details can be found in [10]. The sensors were sealed with O-rings at the top side and mounted in separate measurement chambers (see Fig. 1). During measurement, eight sensors were exposed to different degradation media composed of either:

- pH buffer solution of pH 7 (Titrisol®, Merck KGaA, Germany),
- pH buffer solution of pH 11(Titrisol®, Merck KGaA, Germany),
- pH buffer solution containing the model enzyme lipase from *Rhizomucor miehei* (LipaseRM, Palatase® 20000L, Novozymes A/S, Denmark),
- cell-culture medium,
- supernatants from THP-1-derived macrophages cell cultures without stimulation,
- supernatants from cells stimulated with tumour necrosis factor-alpha (TNF-α),
- supernatants from cells stimulated with lipopolysaccharide from *Escherichia coli* (LPS-EB),
- supernatants from cells stimulated with lipopolysaccharide from *Porphyromonas gingivalis* (LPS-PG).

The stimulation of THP-1-derived macrophages cell cultures was performed to treat secretion of substances that are specifically involved during the activation of the immune system. This was done in order to proof whether substances that are specific for an immune response cause an unintended change of the degradation rate.

The sensor signals were read-out every 60 min by an impedance analyser (IM6, Zahner Elektrik GmbH, Germany). The sensor read-out was performed by means of impedance spectroscopy (IS) and capacitance–voltage (C–V) measurements in the frequency range of $f = 10^0 – 10^6$ Hz (AC signal amplitude 20 mV) in the accumulation region of the field-effect device by applying a DC potential of $V_{Bias} = -2$ V to the Ag/AgCl reference electrode, and within a DC bias range of $V_{Bias} = -2$ V to +2 V at a fixed frequency of $f = 120$ Hz, respectively. A capacitively coupled counter electrode was arranged in parallel to the reference electrode to bypass the impedance of the reference electrode, which would cover the sensor signal in the higher frequency range otherwise. Further details on the read-out methodology are described in [11]. In order to attain the signal of the blank sensor, prior completion of the experiment, the remaining polymer layers were treated with NaOH inducing accelerated degradation.

3. Results and Discussion

Fig. 2 shows the measured sensor capacitance in accumulation region over time for all eight sensors. An increasing concentration of hydroxide ions (increasing pH) and LipaseRM induced a substantial acceleration of the degradation rate compared to degradation in neutral pH buffer solution (Fig. 2a, see also [11,12]). This is indicated by a faster increase of the measured capacitance and completion of degradation after approximately 6 and 24 hours, respectively. In contrast, cell-culture media containing supernatants from unstimulated macrophage cell culture and

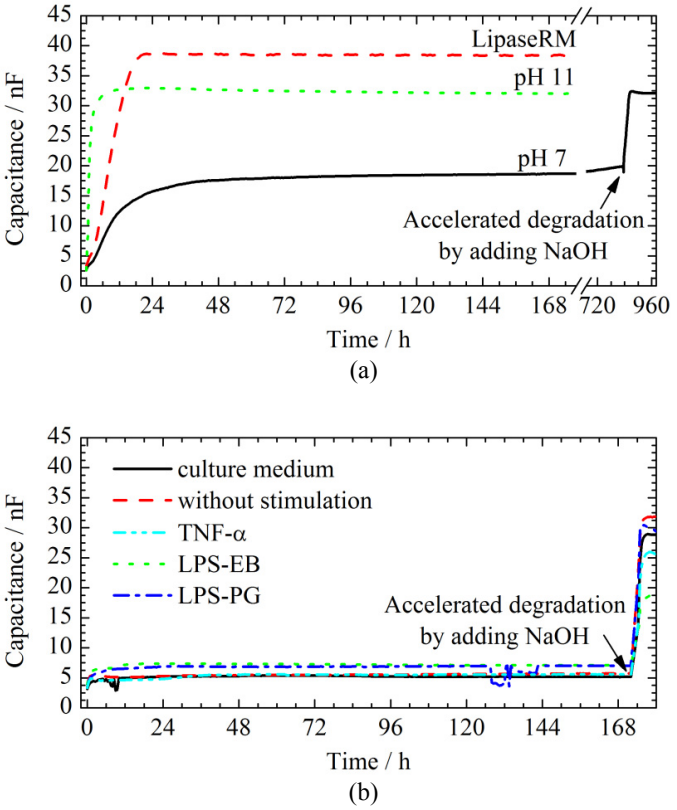

Fig. 2. Time-resolved signal of PMEIS sensors with poly(D,L-lactic acid) exposed to pH buffer and enzyme solutions (a) and cell-culture media (b). The progress of polymer degradation is indicated by an increase in the measured capacitance of the sensors.

with mimicked inflammation by TNF-α, LPS-EB and LPS-PG indicated no significant effect (Fig. 2b) compared to pure culture medium. This implies that activation of macrophages as a consequence of inflammation seems to not influence poly (D,L-lactic acid) degradation *in vivo*. Further experiments are envisaged, however, to study this phenomenon in more detail.

4. Conclusion

In this work, the applicability of a sensor system utilizing capacitive field-effect structures for monitoring of polymer (bio)degradation has been demonstrated. Due to the option of the presented measurement setup to monitor multiple sensor devices in parallel, the system provides distinct potential for degradation studies with high throughput of samples. This was exemplarily investigated on the commercial, biodegradable polymer poly(D,L-lactic acid), which was studied with respect to its degradation rate at different pH values, in the presence of the model enzyme LipaseRM and in the presence of supernatants from THP-1-derived macrophages cell cultures as well as from macrophage cell cultures stimulated with TNF-α, LPS-EB and LPS-PG. The successfully performed experiments and obtained results demonstrate the high potential of sensor-based systems as a novel and promising tool for real-time, *in-situ* electrical monitoring of polymer (bio)degradation.

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